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Bronchial provocation testing can be improved by using dry powder adenosine instead of nebulized AMP

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To the editor:

Airway hyperresponsiveness (AHR) to adenosine has proven to be a good marker for eosinophilic airway inflammation in asthma and can be used to monitor disease activity and therapeutic effectiveness of inhaled corticosteroids (ICS) (1–3). Adenosine is usually administered by nebulization of adenosine monophosphate (AMP) but the highest feasible concentration of AMP often fails to induce sufficient bronchoconstriction in subjects with asthma (4,5). We studied whether this limitation can be resolved by administering adenosine as dry powder formulation. We have previously demonstrated the feasibility of this new bronchial provocation method in a small proof-of-concept study (6). The aim of the present study was to further validate the dry powder adenosine provocation test in a larger cohort of subjects with asthma.

Data were obtained from subjects recruited for the OLIVIA study (clinical trial number: NCT01741285, www.clinicaltrials.gov). Sixty current or ex-smokers with asthma (34 females, 26 males) with FEV_1 ≥50% predicted, who did not use ICS for at least four weeks, underwent provocations with both AMP and dry powder adenosine as baseline measurements on subsequent visits (1–2 weeks apart), in addition to blood sampling, spirometry, body plethysmography, impulse oscillometry (IOS) and multiple breath nitrogen washout (MBNW) measurements. Their mean (±SD) age was 45±12 years and baseline FEV_1 89±16 %predicted.

AMP was administered by nebulization of doubling concentrations (0.04–320 mg/mL). Dry powder adenosine was administered with an investigational inhaler in doubling doses (0.04–80 mg) (6,7). We determined the provocative concentration (PC_{20}) of AMP and dose (PD_{20}) of adenosine causing the forced expiratory volume in 1 s (FEV_1) to drop with 20% by log-

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linear interpolation and assessed which clinical characteristics were predictors of these parameters. Provocation tests were negative if no 20% drop in FEV_1 was reached after administration of the highest concentration/dose and values were censored to 640 mg/mL for PC_{20} AMP and 160 mg for PD_{20} adenosine for analysis. Calculations were performed with the base-2 logarithm (log_2) of PC_{20} AMP and PD_{20} adenosine to reflect the use of doubling dose steps and normalize the distribution.

We calculated the agreement between the two tests with Cohen's kappa and correlation analysis. Correlation analysis was also performed to assess associations between subject baseline characteristics and PC_{20} AMP/PD_{20} adenosine. Associations with a *p*-value <0.20 were considered for multiple linear regression analysis, although per baseline measurement procedure maximally one (the most significant) predictor was included to prevent multicollinearity. Forced entry multiple linear regression analysis was performed to determine which parameters independently predict the airway responses.

Forty subjects reached the predefined 20% drop in FEV_1 on both AMP and adenosine. Ten subjects obtained a positive adenosine test (PD_{20} 5.4–39 mg) but negative AMP test (PC_{20} >320 mg/mL), whereas two subjects had a negative adenosine test (PD_{20} >80 mg) but positive AMP test (PC_{20} 143 and 148 mg/mL). Seven subjects did not reach a 20% drop in FEV_1 on either stimulus. One subject, who had a negative AMP test, experienced severe cough during inhalation of dry powder adenosine, leading to early termination of the test. The total percentage of non-responders was 30% (18 out of 60) for AMP and 15% (9 out of 59) for adenosine. Figure 1A shows PC_{20} AMP and PD_{20} adenosine values, clearly illustrating the higher responder rate to adenosine. PC_{20} AMP and PD_{20} adenosine were strongly correlated

(r_{Sp} = 0.799; Figure 1B), yet had only a moderate agreement (κ = 0.42), mainly due to the larger number of non-responders to AMP.

Baseline variables included in multiple linear regression analysis for PC_{20} AMP were age, smoking status, blood eosinophils, FEV_1, residual volume (RV), and the ventilation heterogeneity of the conductive lung zone (S_{cond}). For PD_{20} adenosine these were age, blood eosinophils, FEV_1, and RV. The models obtained by multiple regression analysis were largely similar for PC_{20} AMP and PD_{20} adenosine with predictive powers of 34% and 30% respectively (Table 1). Only age (AMP and adenosine) and FEV_1 (adenosine) were found to be independent predictors ($p < 0.05$). Age and FEV_1 were positively associated with both PC_{20} AMP and PD_{20} adenosine, whereas blood eosinophils and RV exhibited a trend towards an inverse association.

The present work shows that bronchial provocation with dry powder adenosine is a suitable method for measuring AHR in asthmatic subjects. Moreover, the new test method allowed us to administer higher doses, resulting in fewer false negative test results, while the degree of AHR to dry powder adenosine correlated well with the degree of AHR to nebulized AMP. Despite the greater sensitivity, there were still nine subjects with a negative dry powder adenosine provocation test. Although the order of provocation testing was performed non-randomized with AMP first and dry powder adenosine second one to two weeks later and refractoriness has been shown to occur after AMP provocation (8), we consider any remaining effect one to two weeks later to be unlikely given the findings of Singh et al. (9). Some patients may have developed a component of COPD or asthma-COPD overlap, since this study examined current or former smokers. There was, however, no relationship apparent between measures of airway obstruction at baseline and PD_{20} adenosine (e.g. only two out of

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nine had an FEV_1/FVC ratio <70%) or with their smoking status (four current and five former smokers). Therefore, we expect that increasing the top dose, which was now arbitrarily chosen at 80 mg, could further reduce the number of false negatives and thus increase the test's sensitivity even more. However, it cannot be ruled out that there may actually be subjects with asthma that remain unresponsive to even higher doses inhaled adenosine, which requires further investigation.

The subjects did not appear to react more severely to dry powder adenosine than anticipated from their responsiveness to AMP, indicating that the test is safe to use. Severe cough, a side effect that has been shown to hinder applicability of the mannitol provocation test (10), another indirect measure of AHR, was only reported in one subject. No other side effects were observed.

We previously reported that AHR to AMP is associated with eosinophilic inflammation (1). In the present study, blood eosinophils were included in the prediction models, although their individual contributions were not significant for either PC_{20} AMP or PD_{20} adenosine (p = 0.066 and p = 0.11 respectively). This can be explained by the fact that in the present study we investigated eosinophilic inflammation in blood rather than sputum, by the smaller study population (60 vs. 120 patients (1)) and the non-parametric distribution due to the high number of non-responders, especially to AMP. Alternatively, differences in smoking behavior of the subjects may have played a role. Smoking has been shown to blunt eosinophilic inflammation, demonstrated by lower numbers of eosinophils in sputum and blood of smokers and ex-smokers compared to never-smokers (11). Further studies in never-smokers are therefore warranted.

In conclusion, we have shown that bronchial provocation with dry powder adenosine is a suitable alternative to provocation with nebulized AMP, considering the good agreement between the tests and comparable baseline predictors. Moreover, dry powder adenosine appears to offer an improvement over nebulized AMP, because of its higher sensitivity for less hyperresponsive subjects with asthma.

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Figure 1: (A) Comparison of the PC_{20} AMP and PD_{20} adenosine. The lines indicate the geometric means, * depicts negative test results, which were censored to PC_{20} AMP = 640 mg/mL and PD_{20} adenosine = 160 mg in the analyses. (B) Correlation analysis between PC_{20} AMP and PD_{20} adenosine, showing a strongly significant correlation between the two test results (r_{Sp} = 0.799, $p < 0.001$).

Table 1: Baseline predictors for PC_{20} AMP and PD_{20} adenosine obtained by multiple linear regression analysis.

Dependent variable	Baseline predictor	B	CI 95%	*p*-value	R^2
log_2 PC_{20} AMP	Age (years)	0.111	0.035; 0.187	**0.005**	0.34
	Smoking status	-0.028	-1.82; 1.77	0.98	
	Eos blood (% total)	-0.306	-0.686; 0.074	0.11	
	FEV_1 (%pred)	0.047	-0.011; 0.104	0.11	
	RV (%pred)	-0.018	-0.054; 0.017	0.31	
	S_{cond} (/L)	-1.94	-42.1; 38.2	0.92	
log_2 PD_{20} Adenosine	Age (years)	0.059	0.007; 0.112	**0.027**	0.30
	Eos blood (% total)	-0.244	-0.542; 0.055	0.11	
	FEV_1 (%pred)	0.052	0.009; 0.096	**0.020**	
	RV (%pred)	-0.024	-0.050; 0.002	0.073	

PC_{20}: provocative concentration causing a 20% drop in FEV_1; PD_{20}: provocative dose causing a 20% drop in FEV_1; Eos blood: blood eosinophils as percentage of total leukocytes; FEV_1: forced expiratory volume in 1 s; RV: residual volume; S_{cond}: ventilation heterogeneity of the conductive lung zone.

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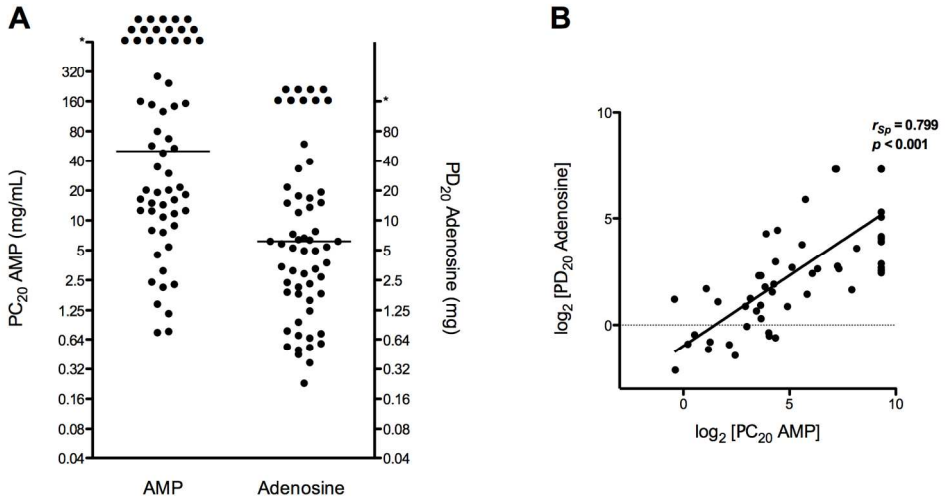

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